A PATHWAY APPROACH TO PREDICTING THYROID HORMONE DISRUPTING ACTIVITY OF CHEMICALS USING IN VITRO, EX VIVO AND IN VIVO ASSAYS

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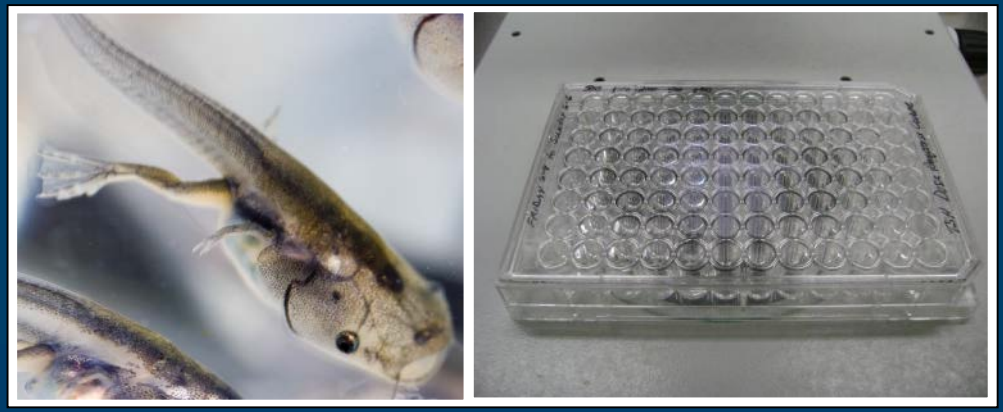


Office of Research and Development
National Health and Environmental Effects Laboratory

Background

- Safe Drinking Water Act & Food Quality Protection Act mandate that USEPA assess chemicals for endocrine activity
  - Many chemicals with limited or no information on thyroid activity

- Need to prioritize what chemicals should be tested

Objectives

- Develop higher throughput, mechanism-based, predictive tools for disruption of thyroid hormone

- Support the development of chemical structure activity relationship (QSAR) models for thyroid hormone disrupting activity = predictive models

Interference with Maintaining Circulating T4 by HPT

Thyroid Peroxidase (TPO):
Tyrosine Iodination & Coupling

Sodium-Iodide Symporter:
Iodide Uptake

Metabolizing Enzyme
Induction / Activity

Liver

T4
Metabolism

Hypothalamus

TRH/CRH

Pituitary

TSH

Thyroid Gland

Iodide

NIS

I + Tyr → MIT

DIT

TPO

DIT

DIT

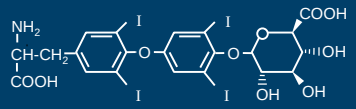
T4

← T4 →

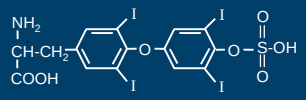
Peripheral Tissues

T4 → T3
T3 → TH receptor
TH responsive genes

Elimination

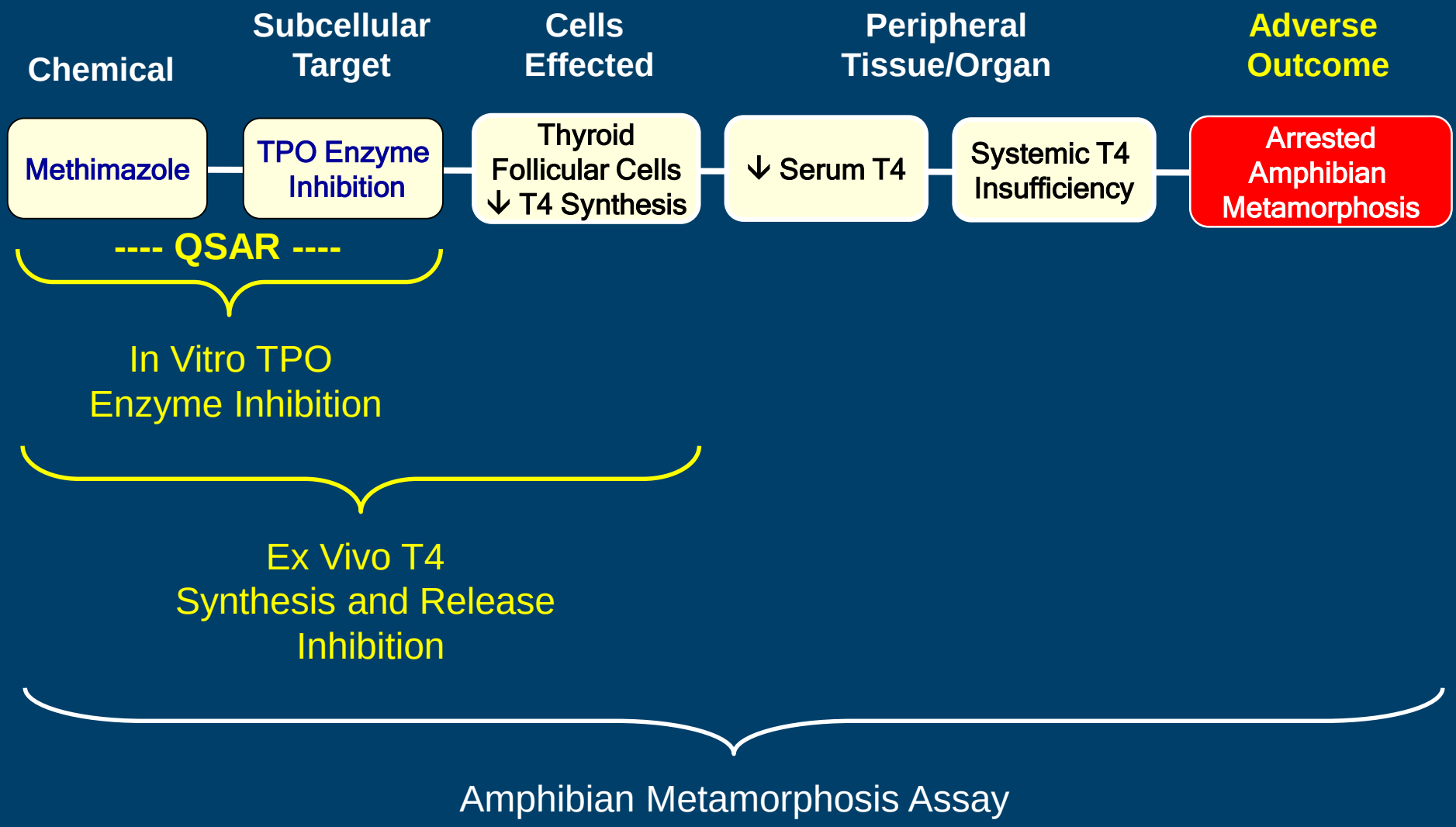


T4-glucuronide



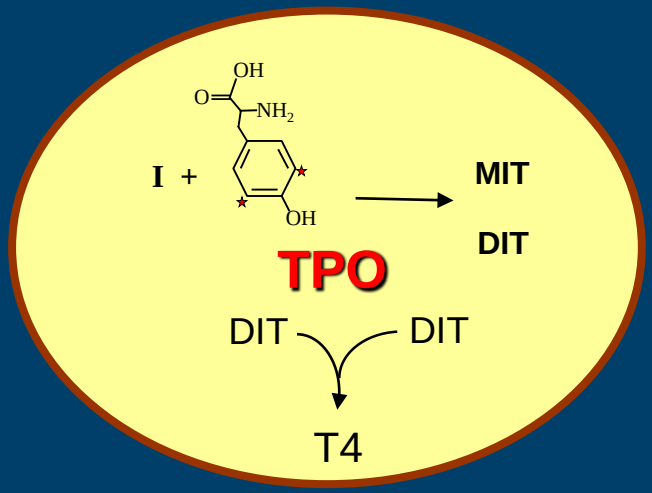
T4-sulfate

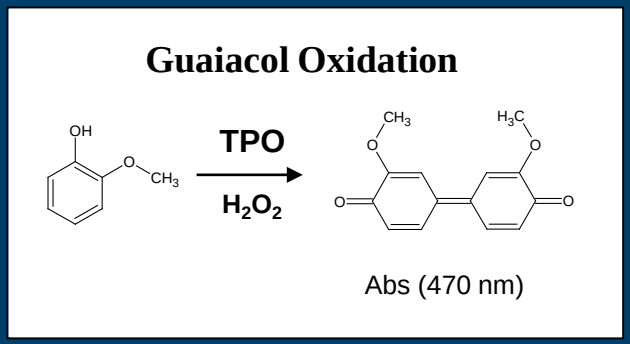
Tiered Assay Approach from Molecular Initiating Event to Adverse Outcome



TPO Inhibition Assay

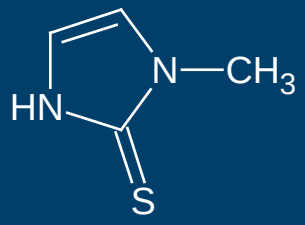
- Prepare microsomes from thyroid glands (porcine)
- In Vitro Assay: 96-well plate
- Colorimetric Endpoint



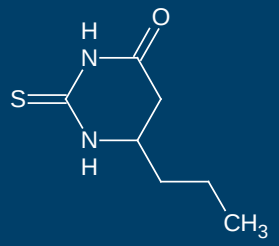


Selection of Chemicals to Test in the In Vitro Assays

- **Test chemicals based upon structural similarity to known active chemicals : model pharmaceuticals**

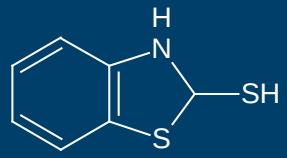


methimazole

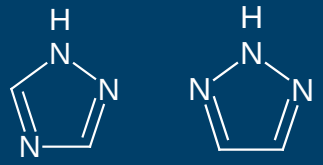


PTU

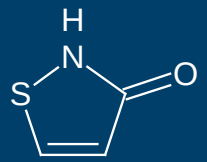
Test chemical classes



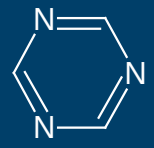
thiazoles and benzothiazoles



triazoles



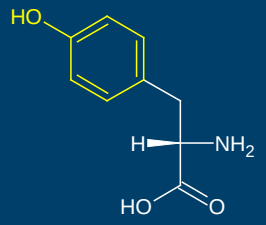
isothiazolinones



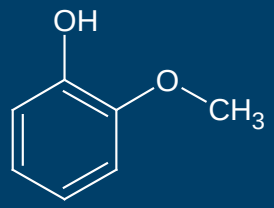
triazines

Selection of Chemicals to Test in the In Vitro Assays

- **Test additional chemicals based upon structural similarity to known TPO substrates: potential competitive inhibitors**
  - Endogenous substrate: tyrosine

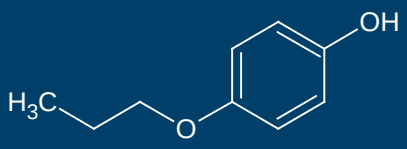


  - TPO enzyme assay substrate: guaiacol (*o*-methoxyphenol)

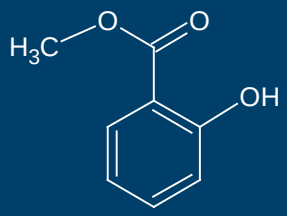


Test Chemical Classes

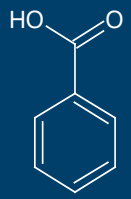
alkoxyphenols



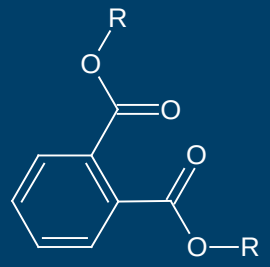
salicylates



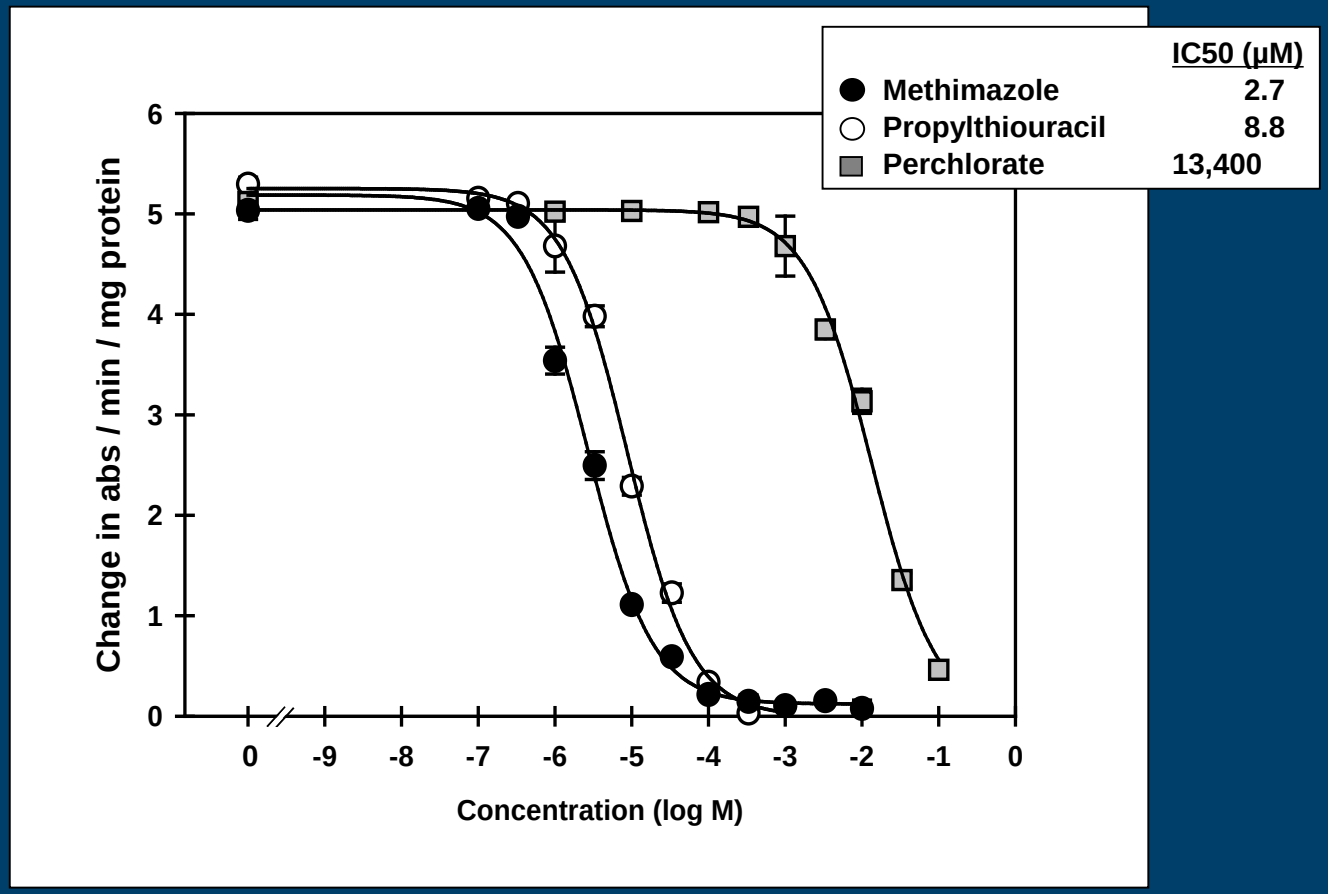
benzoates



phthalates

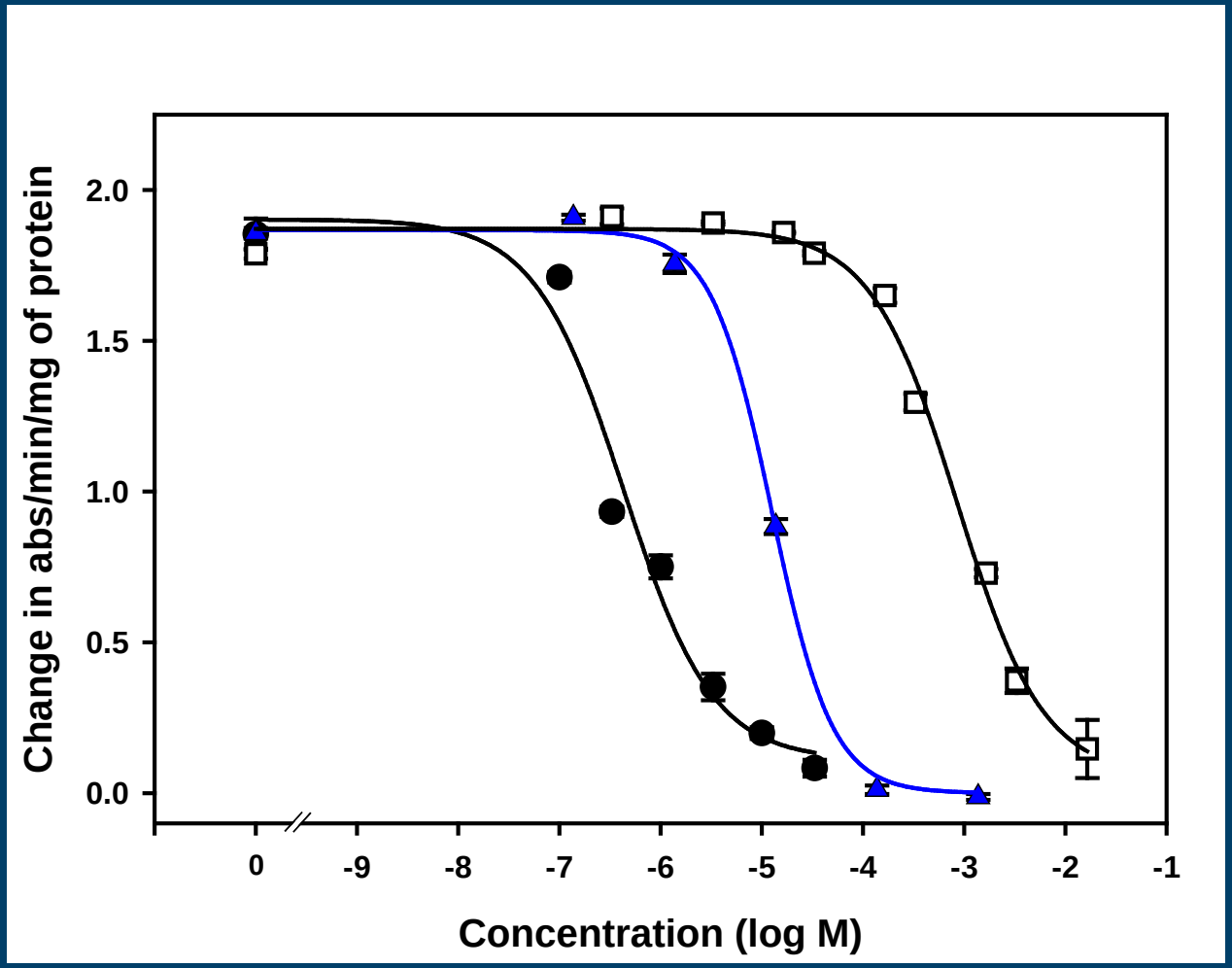


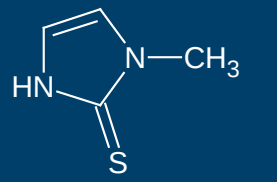
In Vitro TPO Inhibition by Model T4 Synthesis Inhibitors



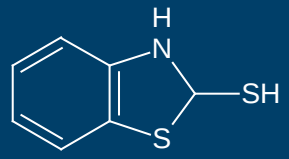
Identification of TPO inhibitors

TPO Inhibition by mercaptobenzothiazole (MBT) and dimethyl hydroxymethylpyrazole (DMP)

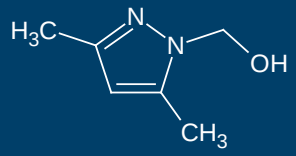




Methimazole

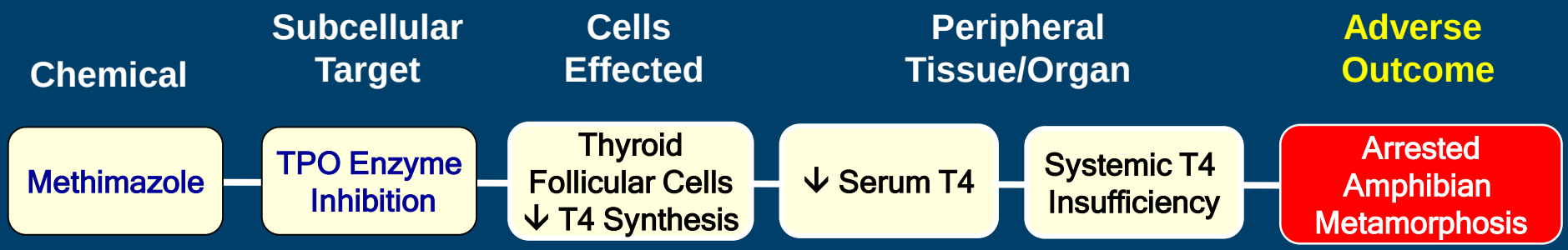


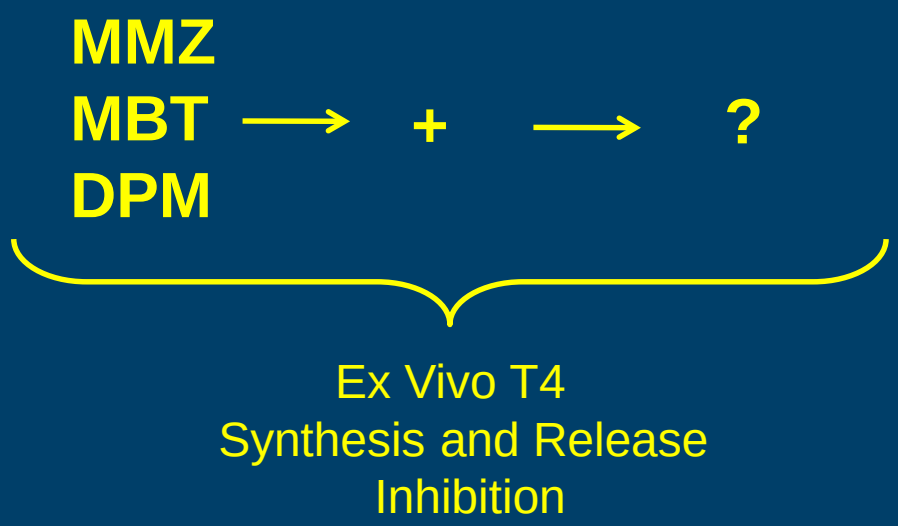
MBT



DMP

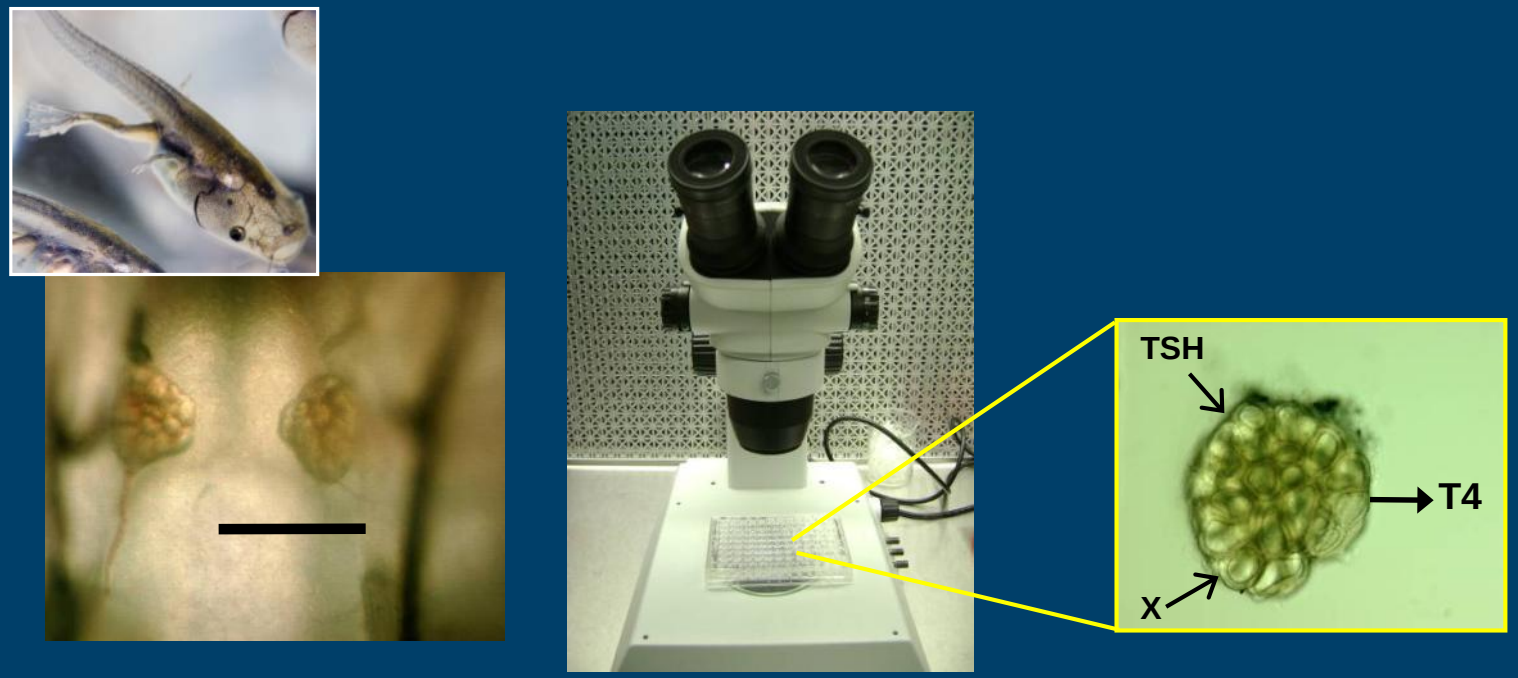
Test at Next Higher Level of Biological Organization





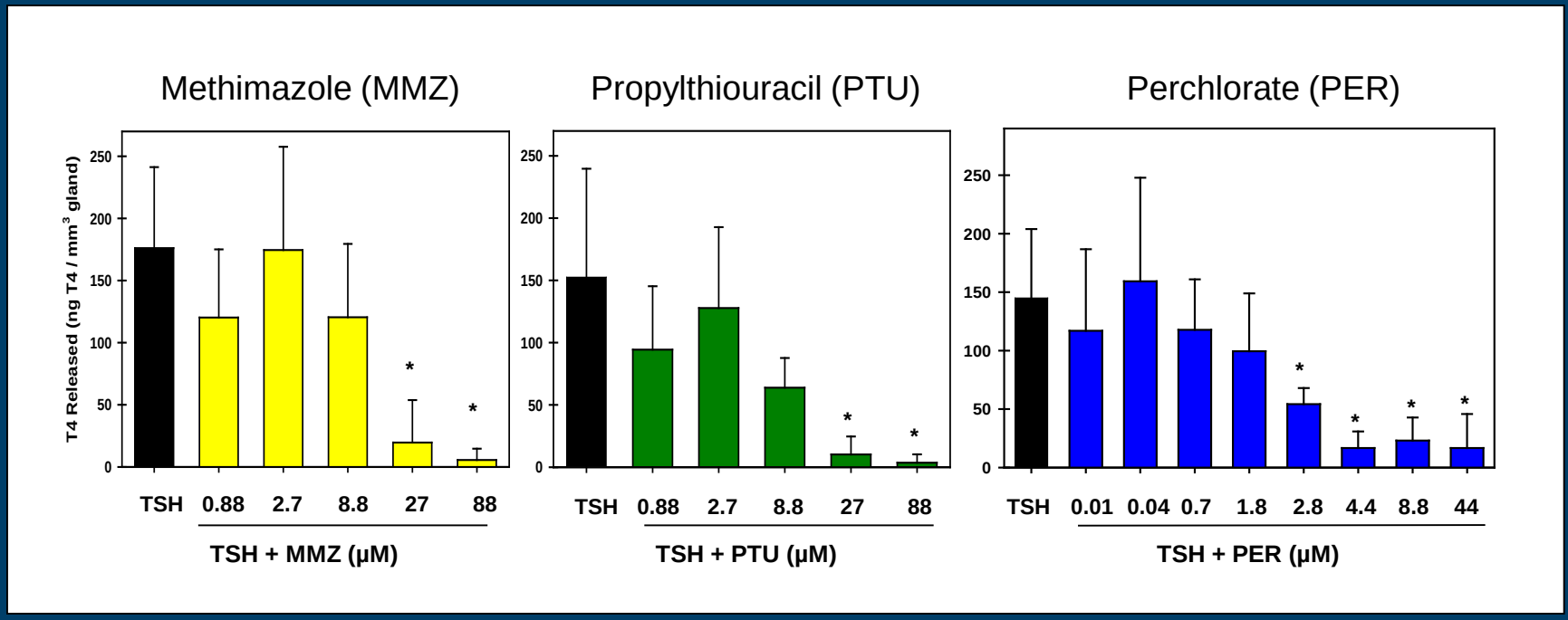
Ex Vivo Assays: Thyroid Explant Cultures

- *X. laevis* explant culture assays
  - Dissect glands from NF stage 59 tadpoles
  - Culture in 96-well plates
  - Inhibition of bTSH stimulated T4 synthesis and release



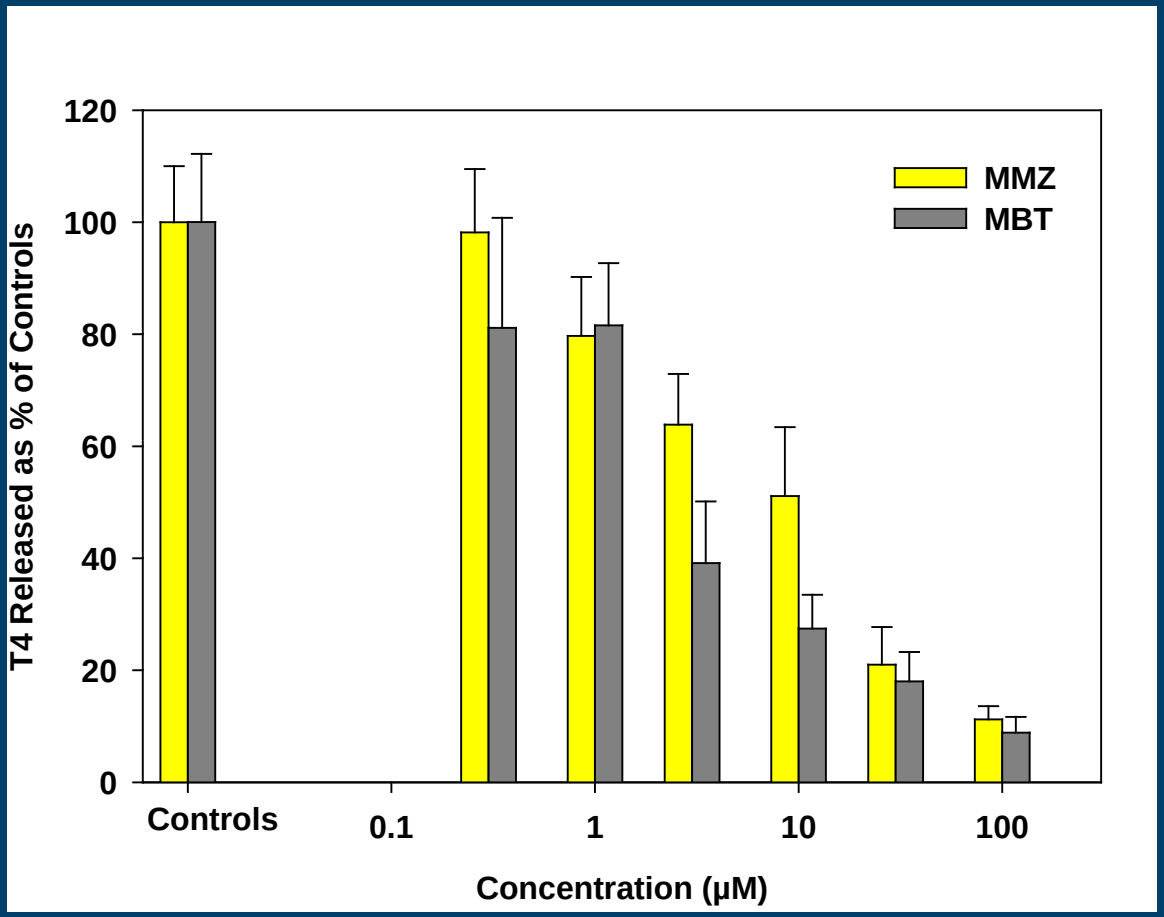
In Vitro Assays: Thyroid Explant Cultures

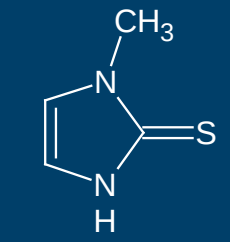
1. Inhibition of T4 Release by Model TH Synthesis Inhibitors

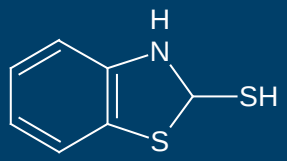


In Vitro Assays: Thyroid Explant Cultures

2. Test positives from TPO inhibition assay



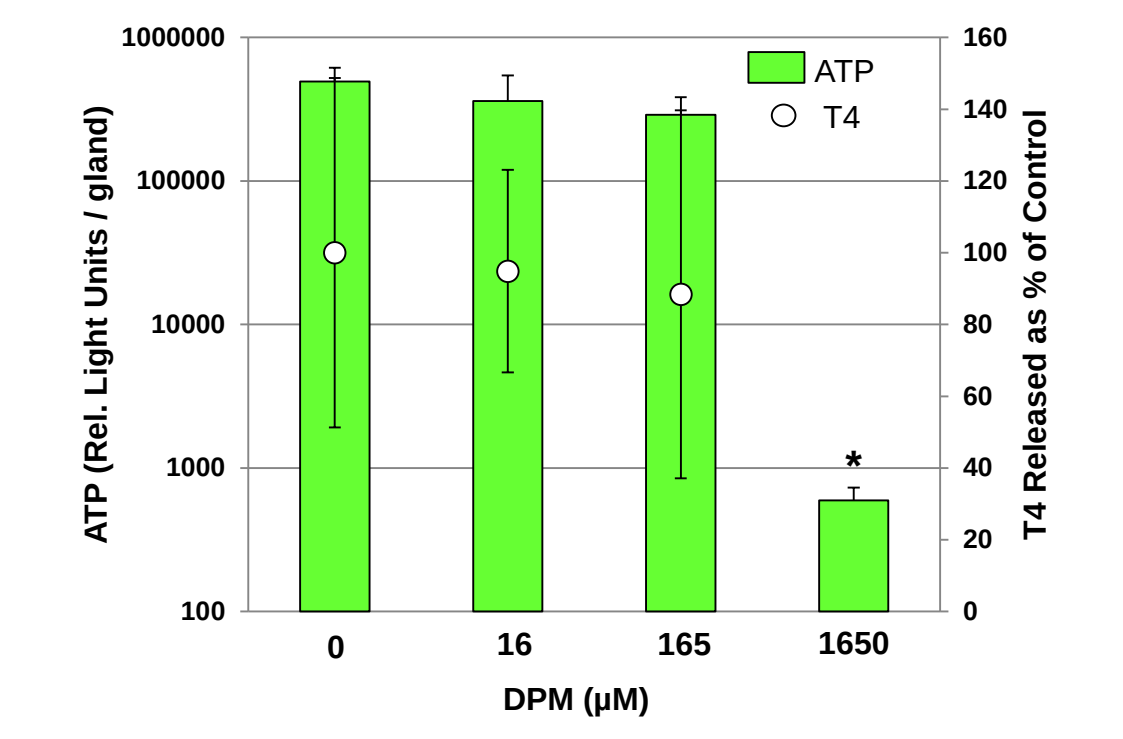


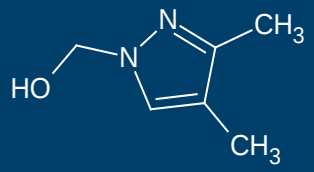


In Vitro Assays: Thyroid Explant Cultures

- **TPO inhibitors may be toxic to the cultured glands**

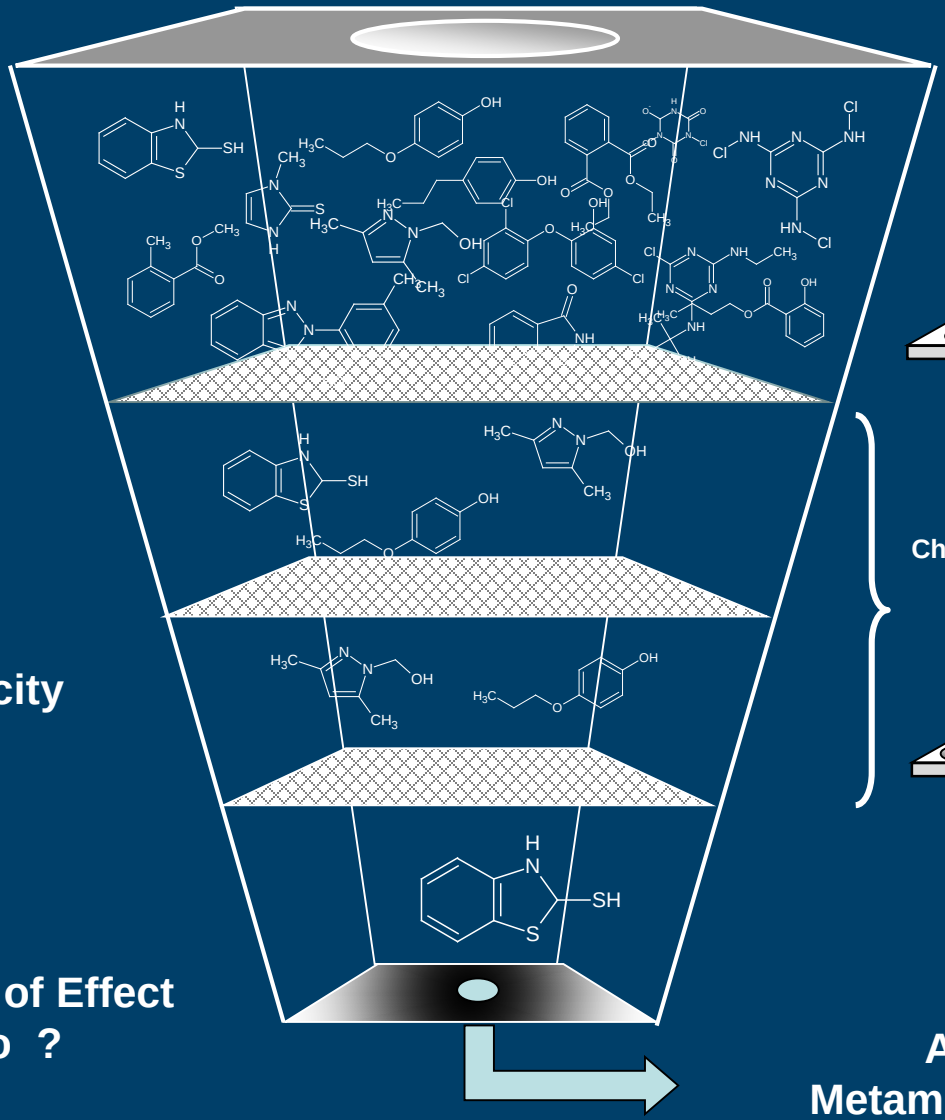
Gland Viability (ATP)



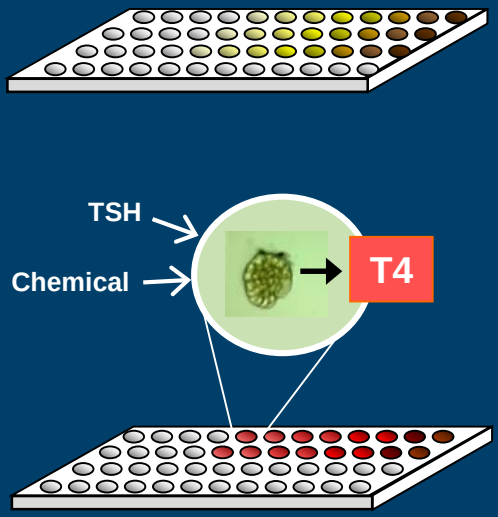


Tiered Assay Approach to Prioritization of Chemicals for Further Testing

Thyroid Peroxidase Inhibition In Vitro



Thyroid Microsomes + Chemical + Substrate



Cytotoxicity Filter

Verification of Effect In Vivo ?

Amphibian Metamorphosis Assay

Amphibian Metamorphosis Assay

- OECD 21d AMA protocol
- Initiate at NF Stage 51
- 21d exposure duration
- continuous flow through waterborne exposure

Endpoints

- Metamorphic Development
- Thyroid Histology

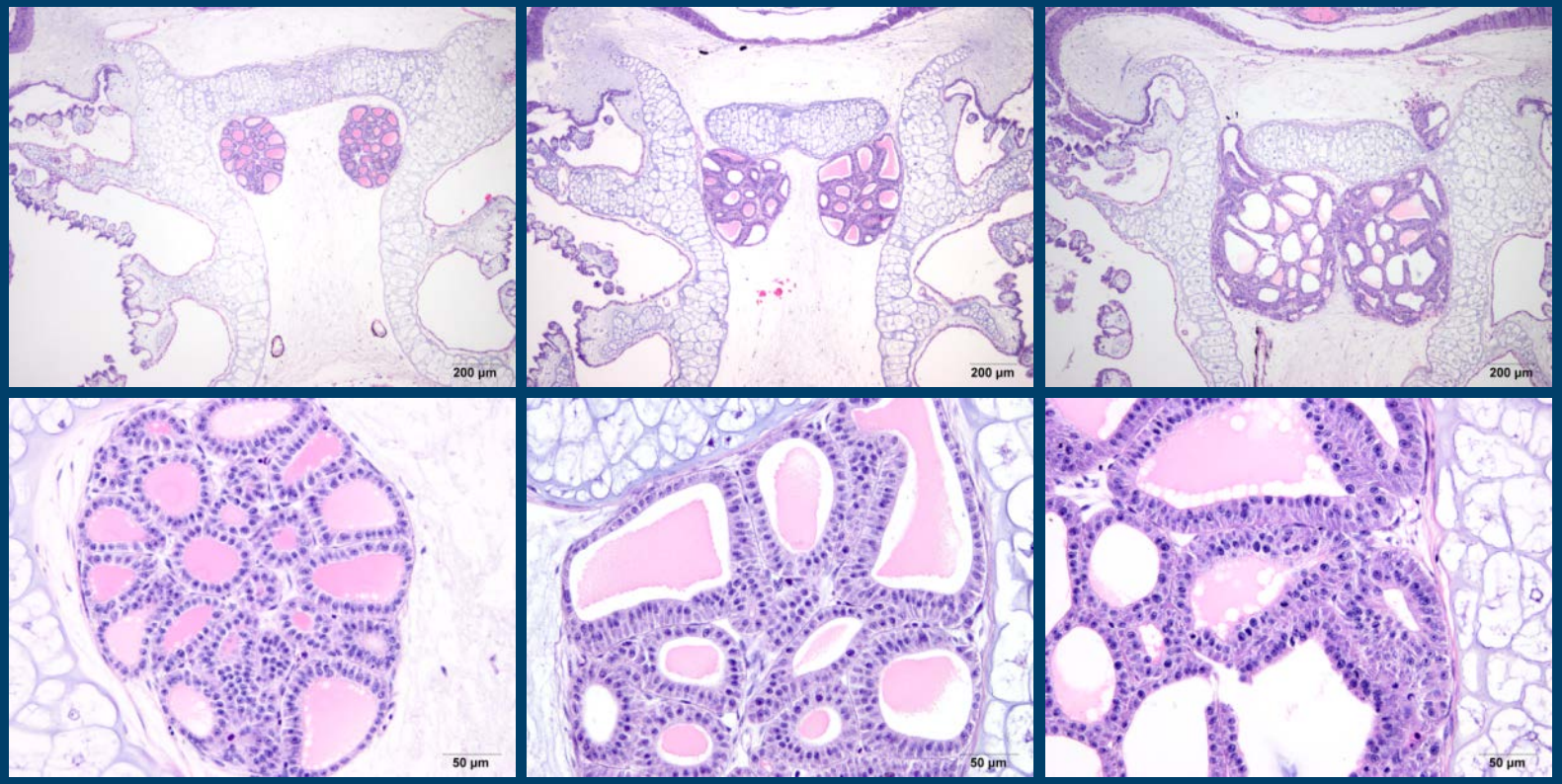
- Thyroidal Iodo-amino Acids by HPLC-ICP/MS
- Serum T4 and T3 by HPLC-ICP/MS
- Sodium Iodide Symporter (NIS) mRNA Expression
- Thyroid Stimulating Hormone by ELISA

MBT Exposure: Tadpole NF Stage Distribution

21 Day MBT Exposure											
[MBT] (µg/L)		Final NF Stage									
		55	56	57	58	59	60	61	62	63	64
0		0	0	0	0	5	38	10	13	32	2
23		0	0	0	0	14	20	9	32	25	0
47		0	0	0	2	15	36	7	27	14	0
109	*	0	2	2	8	17	30	7	30	5	0
214	*	0	2	8	43	37	8	0	2	0	0
435	*	40	33	22	5	0	0	0	0	0	0

MBT Exposure: Thyroid Gland Histology

control | 82 µg/L | 357 µg/L

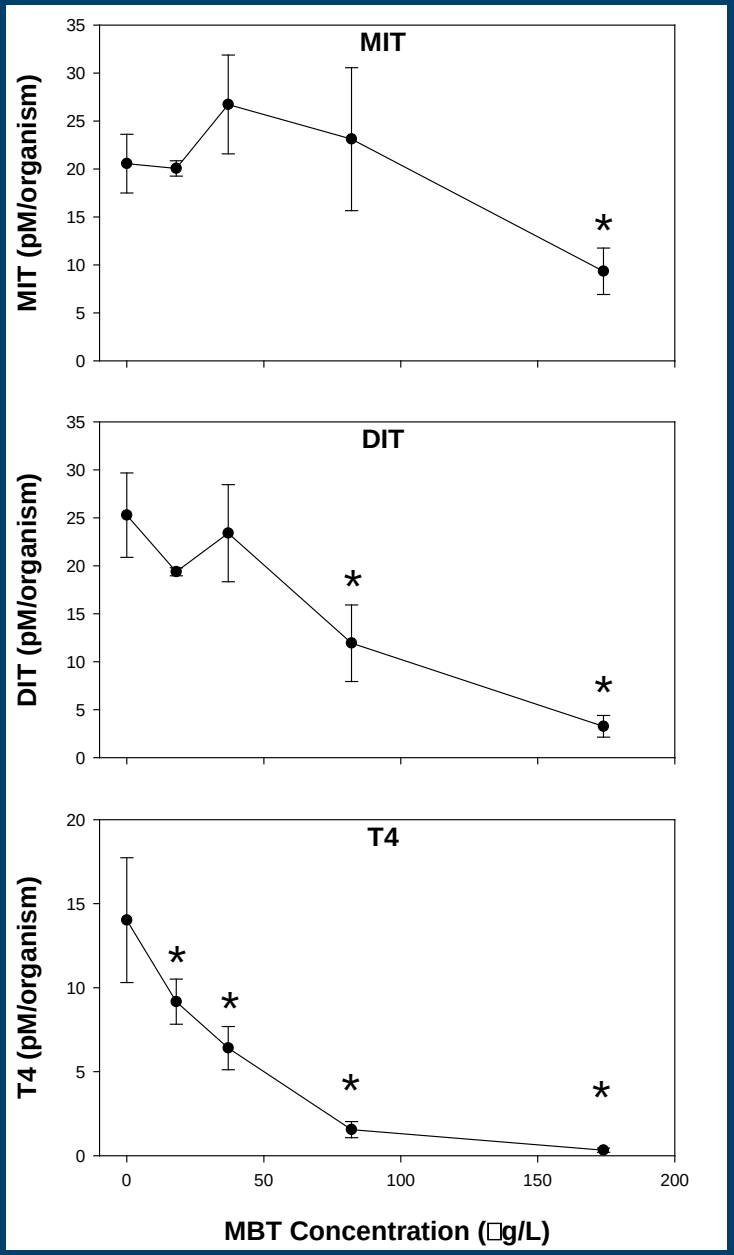


Effects on Thyroidal Hormones

Reduces monoiodination →

Reduces diiodination →

Reduces T4 formation →

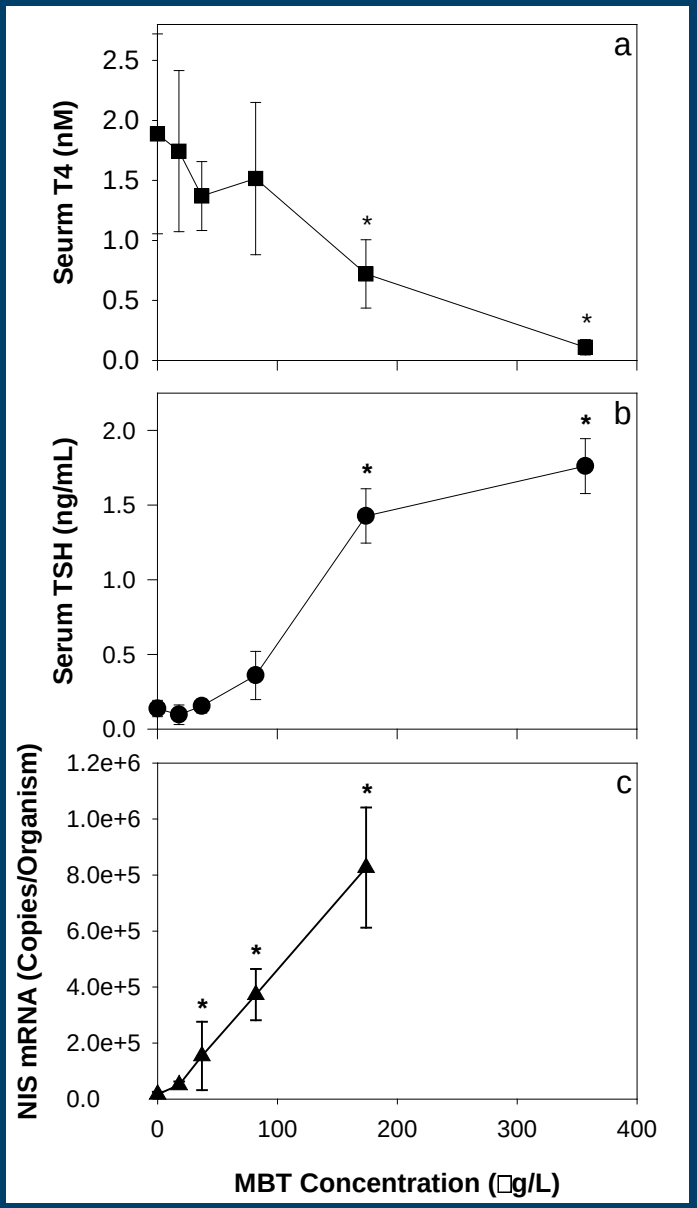


Effect on Circulating Hormones & NIS

Reduces circulating T4 ⟶

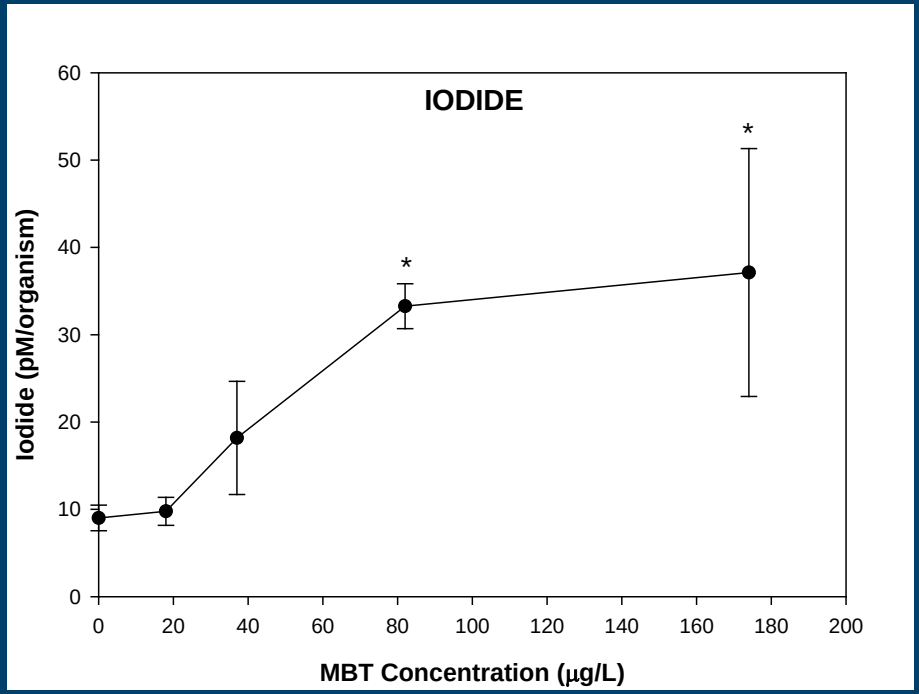
Increases circulating TSH ⟶

Increases thyroidal NIS mRNA ⟶



Effect on Thyroidal Iodide

And…
thyroidal iodide increases due to reduced TH synthesis and increased NIS



Summary and Conclusions

- Developed tiered prioritization approach based upon the specific pathway of TPO inhibition
  - An important mechanism of action in thyroid toxicology
  - Identified specific activity with TPO assay
  - Demonstrated TH reductions in thyroid gland assay
  - Confirmed activity and potency in organismal assay

Identified potent chemical

- *In vivo* results clearly indicate TH synthesis inhibition
- Similar potency to methimazole in vitro/ex vivo
- Very potent chemical in vivo
  - Methimazole effects: 12-25 mg/L
  - MBT: LOEC Histology ≈ 40 ug/L

QUESTIONS ?